Docket Number: P-LG4878

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	Examiner: STRZELECKA, Teresa E.
PADGETT et al.	)	Group Art Unit: 1637
Serial No.: 10/066,390	)	
Filed: February 1, 2002	)	I hereby certify that this correspondence is being transmitted to the USPTO via EFS-Web on May 21, 2008 by /Wayne Fitzmaurice/
For: A Method of Increasing Complementarity in a Heteroduplex	)	Wayne P. Fitzmaurice Reg. No. 58,274

DECLARATION UNDER RULE 132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

May 21, 2008

Dear Sir:

I, Hal S. Padgett, do hereby declare and state:

1. I received a B.S. in Life Sciences in 1988 from the University of Missouri at Rolla and a Ph.D. in Molecular Microbiology and Microbial Pathogenesis in 1996 from Washington University in Saint Louis, Missouri. I joined Large Scale Biology Corporation in 1998. For nine years my responsibilities involved viral vector development and molecular evolution programs at Large Scale Biology Corporation. Since 2007 I have been the Chief Scientist and General Manager of Novici Biotech, LLC.

2. I am a named inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled A Method of Increasing Complementarity in a Heteroduplex.

3. The references below outline the prevailing thought regarding the introduction of multiple nicks in the presence of a DNA mismatch repair system. I conclude that it would be disadvantageous to introduce additional nicks into a heteroduplex DNA in addition to those already introduced by the mismatch repair system, especially in a way that is not coordinated and controlled by the mismatch repair system.

In their paper Mismatch-stimulated killing, Doutriaux et al., suggest that "inactivation of heteroduplexes is the result of a double-strand break produced by overlapping excision tracts initiating on opposite strands". Basically, the exonuclease that starts at a given nick and degrades hundreds or thousands of bases of DNA of that nicked strand would create a double strand break if it were to encounter a nick or gap in the opposite strand.

Borts and Haber point out that in their yeast system, multiple, independent mismatch repair events on a heteroduplex can generate double strand breaks or gaps. They add that "such breaks could be created by converging excision-repair enzymes operating on opposite strands of the same heteroduplex".

The results of Wagner et al., "indicate that undirected mismatch repair can be lethal", and that the mechanism of lethality may involve simultaneous, or nearly simultaneous, attack on both strands at a single mismatch to produce a double-strand break.

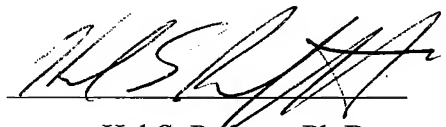
Indeed, Volkov et al., point out that their in vivo mismatch repair-mediated recombination system is improved by eliminating nicks prior to exposure to the DNA repair system. They characterize the presence of additional single stranded nicks in their heteroduplex substrate as a "problem" and showed that by reducing the number of nicks in their heteroduplex substrates, they could improve their system and obtain up to a 7-fold increased yield of chimeric constructs.

In view of the results presented in these references, it can be concluded that the introduction of additional nicks into a heteroduplex molecule, especially without regard to which strand is nicked, will likely diminish the number of intact DNA molecules recovered as well as reduce the proportion of shuffled clones contained in the set of recovered clones.

These observations are relevant to Claims 91-105 the application.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and that the statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, and patent to which this verified statement is directed.

May 21, 2008
Date



Hal S. Padgett, Ph.D.

Attachments:

- Reference Listing

References:

BORTS, R.H. and HABER, J.E., "Meiotic Recombination in Yeast: Alteration by Multiple Heterozygosities", Science 237 (1987) 1459-1465

WAGNER et al., "Involvement of *Escherichia coli* Mismatch Repair in DNA Replication and Recombination", Cold Spring Harb Symp Quant Biol 49 (1984) 611-615

DOUTRIAUX et al., "Mismatch-stimulated killing", Proc Natl Acad Sci USA 83 (1986) 2576-2578

VOLKOV et al. Nucleic Acids Res 27 (1999) e18